Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 5066–5071
Bioorganic & Medicinal Chemistry

Solution conformations of amphidinolide H

Kazutaka Shimbo, Kohei Nozawa, Masashi Tsuda and Jun'ichi Kobayashi*

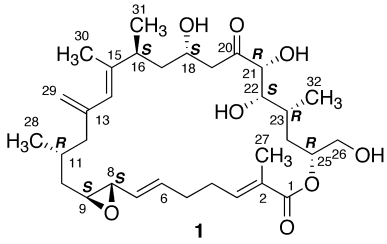
Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Received 22 January 2005; revised 9 April 2005; accepted 11 April 2005
Available online 24 May 2005

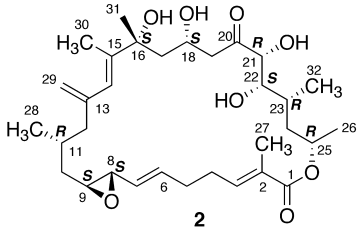
Abstract—Solution conformations of amphidinolide H (**1**), a 26-membered macrolide exhibiting potent cytotoxic and antitumor activity, in $CDCl_3$ and DMSO-d_6 were investigated on the basis of NMR data, distance geometry calculation, and restrained energy minimization. Three-dimensional conformations in $CDCl_3$ were suggested to be close to the X-ray structure of **1**, while those in DMSO-d_6 were indicated to be different from both those in $CDCl_3$ and the X-ray structure.

1. Introduction

Amphidinolides are a series of unique cytotoxic macrolides isolated from marine dinoflagellates *Amphidinium* sp., which were separated from acoel flatworms *Amphiscolops* sp.[1] Amphidinolides H[2] (**1**) and B[3] (**2**), which were initially isolated from a marine dinoflagellates *Amphidinium* sp. (Y-25 and Y-5 strains, respectively), are 26-membered macrolides possessing unique structural features such as an allyl epoxide and vicinally located one-carbon branches. The relative and absolute stereochemistries of amphidinolide B (**2**) have been established by Shimizu's[4] and our groups,[5] respectively. Recently, we have separated a strain (Y-72) of the genus *Amphidinium* producing a relatively large amount of amphidinolide H (**1**), and determined the absolute stereochemistry of **1** on the basis of the X-ray diffraction analysis and synthesis of a degradation product.[6] The structures including the absolute stereochemistry of **1** and **2** are similar to each other, and the only different point is the position of a hydroxyl group, which is attached to C-16 in **2**, while it is on C-26 in **1**. Furthermore, four amphidinolide H-congeners,[7] amphidinolide H2, H3, H4, and H5, and 27-membered macrolides related to **1**, amphidinolides G,[2] G2,[7] G3,[7] and L[8] and stereoisomers of **2**, amphidinolides D[9] (equal to B2[4]) and B3,[4] were isolated from the *Amphidinium* dinoflagellates. Among these macrolides, amphidinolides H (**1**) and B (**2**) exhibited potent cytotoxicity (IC_{50} 0.0045–0.00014 μg/mL) against cultured tumor cells in vitro, and amphidinolide H (**1**) showed antitumor activity in vivo. Recently, it was indicated that one of the mechanism of action for the potent cytotoxicity of **1** was due to bind to actin subdomain 4 covalently.[10] On the other hand, amphidinolide B (**2**) was found to activate contractile system of skeletal muscles through enhancing the actomyosin-ATPase activity.[11]



1



2

In this paper we described the solution conformations of amphidinolide H (**1**) deduced from extensive NMR data, distance geometry calculation, and restrained energy minimization.

Keywords: Solution conformation; Cytotoxic; Macrolide.
* Corresponding author. Tel.: +81 11 706 4985; fax: +81 11 706 4989; e-mail: jkobay@pharm.hokudai.ac.jp

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.04.021

2. Results and discussion

2.1. X-ray structures of amphidinolides H (1) and B (2)

Solid-state structures of amphidinolides H[6] (**1**) and B[4] (**2**) obtained by X-ray crystalline analysis were close to each other, as the overlay of backbone structures was shown in Figure 1. Both structures had an intramolecular hydrogen bond (**1**; 1.99 Å, **2**; 2.02 Å) between the hydroxyl group at C-21 and the epoxide oxygen atom, and their macrocyclic skeletons overlapped well each other.

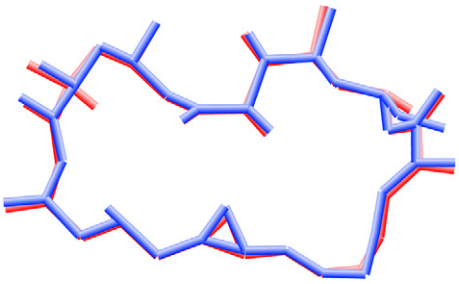


Figure 1. Overlay of X-ray structures of amphidinolides H (**1**, blue) and B (**2**, red).

Table 1. Comparison of conformational groups **A**–**D** of amphidinolide H (**1**) in $CDCl_3$ based on structural parameters

Parameters	A	B	C	D
No. of conformers	8	2	5	1
Energy (kcal/mol)[a]	214.756	217.41	218.676	218.309
RMS NOE	0.223	0.236	0.225	0.234
RMSD	0.02	0.01	0.03	

[a] Mean value except for **D**.

To estimate three-dimensional structure of amphidinolide H (**1**) in $CDCl_3$, the solution conformations were evaluated by distance geometry calculation using SYBYL program.[12] Amphidinolide H (**1**) was subjected to conformational analysis using distance constraints based on intensity of the 86 cross-peaks obtained from the NOESY spectrum (mixing time, 600 ms) in $CDCl_3$. Each of 100 conformers generated by the distance geometry method in $CDCl_3$ was calculated by using simulated annealing protocol and minimization, and finally 71 conformers were obtained. Sixteen of 71 conformations existed in the energy range of 10 kcal/mol from the lowest-energy conformer. Clustering of the 16 conformations based on the structural similarity resulted in the four conformational families **A**–**D** (Tables 1 and 2) and the lowest-energy conformations in each family are shown in Figure 2. The conformational families **A**–**D** comprised eight, two, five, and one conformations, respectively, and these families were distinguished by difference of the dihedral angle of the *S*-*cis*-diene moiety (C-29–C-13–C-14–C-15) and orientation of the ester carbonyl group at C-1. The dihedral angles of the *S*-*cis*-diene moiety for **A** and **B** were ca. −40°, while those for **C** and **D** were ca. +30°. On the other hand, the ester carbonyl groups for **A** and **C** were oriented outside of the macrocyclic ring, while those for **B** and **D** were

Table 2. Summary of dihedral angles of crystal structure and solution conformations for amphidinolide H (**1**)

Bond position	Dihedral angles (°)								
	Crystal structure	Solution conformation							
		$CDCl_3$				DMSO-d_6			
		A	B	C	D	E	F	G	H
O(1) C-1 C-2 C-3	−19.8(4)	−13.9	−155.2	−13.3	137.2	4.5	167.6	155.2	166.8
O(1) C-1 C-2 C-27	163.1(3)	165.9	24.8	166.8	−43	−176.1	−12.9	−23.6	−12.2
O(1) C-25 C-24 C-23	68.8(3)	72	113.2	67.9	57.9	166.3	159.8	163.6	162.1
O(1) C-25 C-26 O(8)	−62.6(3)	−45.5	−50.7	−56.8	−50.5	54.0	87.5	53.8	54.9
O(2) C-1 O(1) C-25	0.4(4)	8.6	2.7	9.2	−7.9	14.1	−5.6	14.7	14.8
O(2) C-1 C-2 C-3	159.9(3)	165.4	24.9	165.5	−43.5	−174.9	−13.6	−24.0	−12.7
O(2) C-1 C-2 C-27	−17.2(4)	−14.8	−155	−14.4	136.3	4.5	166.0	157.3	168.4
O(3) C-8 C-7 C-6	−136.0(3)	−131.2	−110.7	−115.7	−122.8	−135.8	−130.5	−135.0	−124.2
O(3) C-8 C-9 C-10	102.4(3)	98.4	99.7	104.3	98.1	114.4	114.4	113.2	113.5
O(3) C-9 C-8 C-7	102.0(3)	97.6	93.5	87.2	80.9	110.1	108.2	109.0	106.4
O(3) C-9 C-10 C-11	−71.4(3)	−63.9	−66.5	−87.3	−64.6	−135.1	−134.5	−132.4	−134.5
O(4) C-18 C-17 C-16	173.8(2)	−178.8	−175.3	−166	−165.6	87.0	82.8	175.9	−140.6
O(4) C-18 C-19 C-20	60.5(3)	66.9	63.4	56.4	49.7	50.7	56.1	40.5	75.2
O(5) C-20 C-19 C-18	49.1(3)	10.5	22	6.9	6.4	−137.0	−139.7	−138.9	132.2
O(5) C-20 C-21 O(6)	10.7(4)	13.4	5	5.7	8.1	108.9	102.4	97.0	137.0
O(5) C-20 C-21 C-22	−110.8(3)	−106.5	−116	−116.0	−117.5	−14.8	−19.8	−25.2	17.6
O(6) C-21 C-20 C-19	−169.1(2)	−166.6	−173.9	−175.2	−172.7	−72.3	−79.3	−83.9	−43.9
O(6) C-21 C-22 O(7)	−73.8(3)	−73.5	−80.2	−82.7	−85.6	−51.1	−40.0	−64.8	−47.8
O(6) C-21 C-22 C-23	47.5(3)	52.7	46	42.5	40.9	73.5	85.5	61.1	76.9
O(7) C-22 C-21 C-20	49.5(3)	45.7	39.9	37.9	37.8	74.6	82.6	59.9	70.6
O(7) C-22 C-23 C-24	−174.6(2)	−169.4	−168	−174.2	−175.6	−176.7	−171.3	178.7	178.8
O(7) C-22 C-23 C-32	−51.7(3)	−44.1	−44.1	−47.5	−48.5	−47.6	−42.7	−50.8	−51.3
O(8) C-26 C-25 C-24	−178.7(2)	−173.8	178.9	175.5	171.8	−62.1	−42.9	−64.1	−64.3
C-1 O(1) C-25 C-24	−122.2(3)	−165.7	−60.6	−162.4	64.3	−171.2	66.0	−173.8	−171.8
C-1 O(1) C-25 C-26	119.5(3)	67.4	173.5	70.6	−71.9	69.1	−64.4	66.3	67.4

(*continued on next page*)

Table 2 (*continued*)

Bond position	Dihedral angles (°)								
	Crystal structure	Solution conformation							
		$CDCl_3$				DMSO-d_6			
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
C-1 C-2 C-3 C-4	−177.1(3)	179.9	−179.6	−179.5	178.7	176.7	176.5	−178.2	−178.0
C-2 C-1 O(1) C-25	−179.9(2)	−172.1	−177.2	−172.1	171.4	−165.2	173.3	−164.4	−164.7
C-2 C-3 C-4 C-5	−130.1(3)	157.9	173.5	156.1	150.3	111.4	105.8	−153.0	−159.8
C-3 C-4 C-5 C-6	−62.1(4)	67.8	64.4	69.7	67.6	−75.3	−84.7	59.3	65.0
C-4 C-3 C-2 C-27	−0.4(5)	0.1	0.4	0.3	−1.1	−2.7	−3.0	0.5	1.0
C-4 C-5 C-6 C-7	−30.2(5)	−147.7	−145.9	−148.5	−148.8	125.4	126.0	33.0	17.0
C-5 C-6 C-7 C-8	−179.0(3)	178.4	178.2	179	176.8	−179.5	179.6	179.8	178.9
C-6 C-7 C-8 C-9	156.5(3)	167.2	−171.6	−174.5	−179.1	159.1	164.2	160.4	170.9
C-7 C-8 O(3) C-9	−113.0(3)	−109.6	−113.6	−117.9	−121	−103.7	−105.8	−103.6	−107.0
C-7 C-8 C-9 C-10	−155.6(3)	−164	−166.8	−168.5	179	−135.5	−137.5	−137.7	−140.0
C-8 O(3) C-9 C-10	−115.8(3)	−110.9	−109.9	−105.5	−110.5	−90.9	−90.5	−92.8	−92.0
C-8 C-9 C-10 C-11	−141.4(3)	−125.9	−129	−150.2	−126.4	165.9	166.6	167.8	166.0
C-9 C-10 C-11 C-12	170.4(3)	169.9	169.9	164.7	162.6	56.3	55.1	54.0	56.5
C-9 C-10 C-11 C-28	−64.8(3)	−63.4	−63.6	−69.8	−71.9	−174.9	−176.2	−177.1	−174.6
C-10 C-11 C-12 C-13	170.4(3)	176.5	178.8	179.7	177.8	−169.3	−172.9	−167.8	−160.8
C-11 C-12 C-13 C-14	61.8(3)	75	77.7	44.3	39.2	32.4	27.0	28.6	35.9
C-11 C-12 C-13 C-29	−119.7(3)	−102.1	−99.8	−139.9	−144.8	−151.3	−156.3	−154.8	−148.3
C-12 C-13 C-14 C-15	−146.3(3)	147.9	144.6	−155.3	−149.9	−131.3	−132.6	−138.4	−142.5
C-13 C-12 C-11 C-28	65.7(3)	49.2	52	53.6	51.4	64.5	61.4	66.2	72.7
C-13 C-14 C-15 C-16	−178.8(3)	174.4	174.3	−177.2	−178	178.8	177.8	177.5	179.1
C-13 C-14 C-15 C-30	0.1(5)	−4.6	−4.7	5.2	4.7	0.4	−0.1	0.1	0.9
C-14 C-15 C-16 C-17	−123.8(3)	−68.1	−68.8	−84	−84.4	−74.7	−77.2	−84.2	−84.6
C-14 C-15 C-16 C-31	112.7(3)	167.3	166.6	151.6	151	151.3	148.6	145.6	143.7
C-15 C-14 C-13 C-29	35.4(5)	−34.9	−37.9	28.9	34.1	52.7	50.9	45.2	42.0
C-15 C-16 C-17 C-18	49.9(4)	69	71.9	61.6	74.6	160.8	160.0	156.0	167.7
C-16 C-17 C-18 C-19	55.7(4)	59.7	60.2	70.6	69.4	−40.2	−44.9	53.8	90.9
C-17 C-16 C-15 C-30	57.2(3)	111	110.2	93.7	93.1	103.8	100.8	93.2	93.7
C-17 C-18 C-19 C-20	−178.4(2)	−171.6	−172.7	179.4	174.3	177.3	−176.6	161.3	−159.6
C-18 C-17 C-16 C-31	174.2(3)	−163.5	−160.8	−171	−158.4	−64.0	−64.3	−74.3	−59.6
C-18 C-19 C-20 C-21	−131.2(3)	−169.5	−159.1	−172.1	−172.9	44.2	42.0	42.0	−46.9
C-19 C-20 C-21 C-22	69.5(3)	73.5	65.1	63.7	61.7	164.0	158.5	153.9	−163.9
C-20 C-21 C-22 C-23	170.8 (2)	172	166.2	163.1	164.3	−160.8	−151.9	−174.2	−164.8
C-21 C-22 C-23 C-24	63.3(3)	65	66.1	60.7	58	57.1	62.6	51.1	54.0
C-21 C-22 C-23 C-32	173.7(3)	−169.7	−170	−172.6	−175	−173.8	−168.8	−178.4	−176.2
C-22 C-23 C-24 C-25	161.2(2)	−156.4	−163.6	−153.2	−148.8	−113.0	−137.2	−108.4	−112.5
C-23 C-24 C-25 C-26	−174.8(2)	−158.5	−121.9	−162.7	−166.1	−74.6	−69.6	−76.6	−76.6
C-25 C-24 C-23 C-32	76.2(3)	78.7	73	80.7	84.5	121.3	97.9	124.6	121.2
C-30 C-15 C-16 C-31	−66.2(3)	−13.6	−14.4	−30.7	−31.5	−30.2	−33.4	−36.9	−38.0

oriented inside of the ring. Although lengths of the hydrogen bond between O(O6)H and epoxide O(O3) for **A**, **C**, and **D** were 1.7–1.8 Å that for **B** was ca. 2.7 Å. The Boltzmann distribution of the obtained conformers suggested that amphidinolide H (**1**) existed as an **A**-type conformation in $CDCl_3$.

The five lowest-energy conformations (total energy; 213.406–214.434 kcal/mol) of amphidinolide H (**1**) in $CDCl_3$ are shown as an overlay in Figures 3 and 4 shows superimposition between the calculated global energy minimum structure and the X-ray structure of **1**. The mean RMSD (root-mean-square deviation) of NOE restraint violations for the five conformers was 0.223(0) Å, the mean RMSD value of distances of these conformers from the X-ray structure showed 0.85(2) Å. The backbone of the global energy minimum structure was similar to the X-ray structure of **1**, while the solution conformations differed slightly from the X-ray structure in terms of lengths of two hydrogen bonds. All these five conformers had an intramolecular hydrogen bond [1.76(1) Å] between the hydroxyl group at C-21 and the epoxide oxygen atom, while that of the X-ray structure was 1.99 Å. The intramolecular hydrogen bond between the hydroxyl group at C-22 and the oxygen atom at C-18, observed for the X-ray structure (1.92 Å), was weak or not observed for the solution conformations (>3.3 Å). In this crystalline structure, it revealed that the *S-cis* diene portion at C-15–C-14–C-13–C-29 was remarkably twisted [torsion angle; −35.6(5)°], while the torsion angle of the global minimum solution conformer was −34.2(6)°.

The solution conformations in DMSO-d_6 were obtained by the same method described above using 74 ROESY correlations to afford 387 conformers from 500 conformers generated by the distance geometry method through simulated annealing protocol and minimization. Sixty seven of 387 conformations existed in the energy range of 5 kcal/mol from the lowest-energy

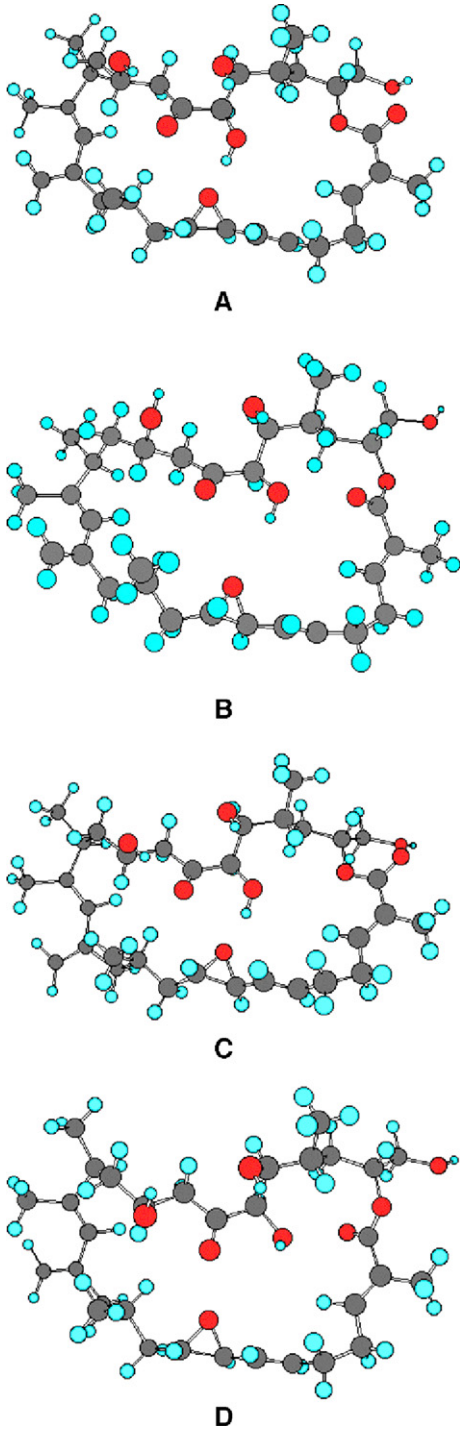


Figure 2. Low-energy conformations of the four confomational families **A**–**D** of amphidinolide H (**1**) calculated by distance geometry analysis based on NOESY data in $CDCl_3$.

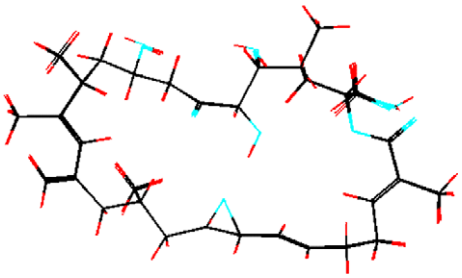


Figure 3. Overlay of the five **A**-type conformations of amphidinolide H (**1**) calculated by distance geometry calculation in $CDCl_3$.

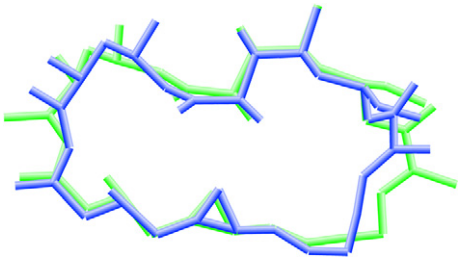


Figure 4. Overlay of the most stable conformations (green) in $CDCl_3$ and X-ray structure (blue) of amphidinolide H (**1**).

Table 3. Comparison of conformational groups **E**–**H** of amphidinolide H (**1**) in DMSO-d_6 based on structural parameters

Parameters	E	F	G	H
No. of conformers	7	8	9	32
Energy (kcal/mol)	264.505	264.01	264.444	265.17
RMS NOE	0.38	0.377	0.381	0.381
RMSD	0.002	0.002	0.002	0.001

conformer. Clustering of these conformations based on the structural similarity resulted in the four main conformational families **E**–**H** (Tables 2 and 3) and the lowest-energy conformations in each family are shown in Figure 5. The conformational families **E**–**H**, which comprised 7, 8, 9, and 32 conformers, respectively, were discriminated by five dihedral angles for O-1–C-1–C-2–C-3, C-3–C-4–C‘-5–C-6, C-4–C-5–C-6–C-7, O-4–C-18–C-17–C-16, and C-16–C-17–C-18–C-19. The dihedral angle for O-1–C-1–C-2–C-3 in conformer **E** was 174.9°, indicating the presence of *S trans*-dienone moiety. On the other hand, other three conformers revealed to have *S-cis*-dienone moiety (**F**: −13.6°, **G**: −24.0°, and **H**: −12.7°). Dihedral angles for C-3–C-4–C-5–C-6 and C-4–C-5–C-6–C-7 in **E** and **F** were ca. 80° and 125°, respectively, while those in **G** and **H** were ca. 60° and 17°–33°, respectively. These results suggested that the carbon chains for conformers **E** and **F** and conformers **G** and **H** turned at C-4 and C-5, respectively. Zig-zag conformation for C-16–C-17–C-18–C-19 in conformers **E** and **F** was deduced from dihedral angles O-4–C-18–C-17–C-16 (87.0° and 82.8°, respectively), and C-16–C-17–C-18–C-19 (−40.2° and −44.9°, respectively). Conformers **G** and **H** consisted of the twisted conformation at C-16–C-17–C-18–C-19, considered from dihedral angles O-4–C-18–C-17–C-16 (175.9° and −140.6°, respectively), and C-16–C-17–C-18–C-19 (−53.8° and 90.9°, respectively). The Boltzmann distribution of the obtained conformers suggested that amphidinolide H (**1**) existed as an **E**-type conformation in DMSO-d_6 Overlay of the seven **E**-type conformations (total energy; 262.0195–266.7302 kcal/mol) of amphidinolide H (**1**) was shown in Figure 6. The presence of three intramolecular hydrogen bonds between (O4)H and O6, between O5 and (O6)H, and between O5 and

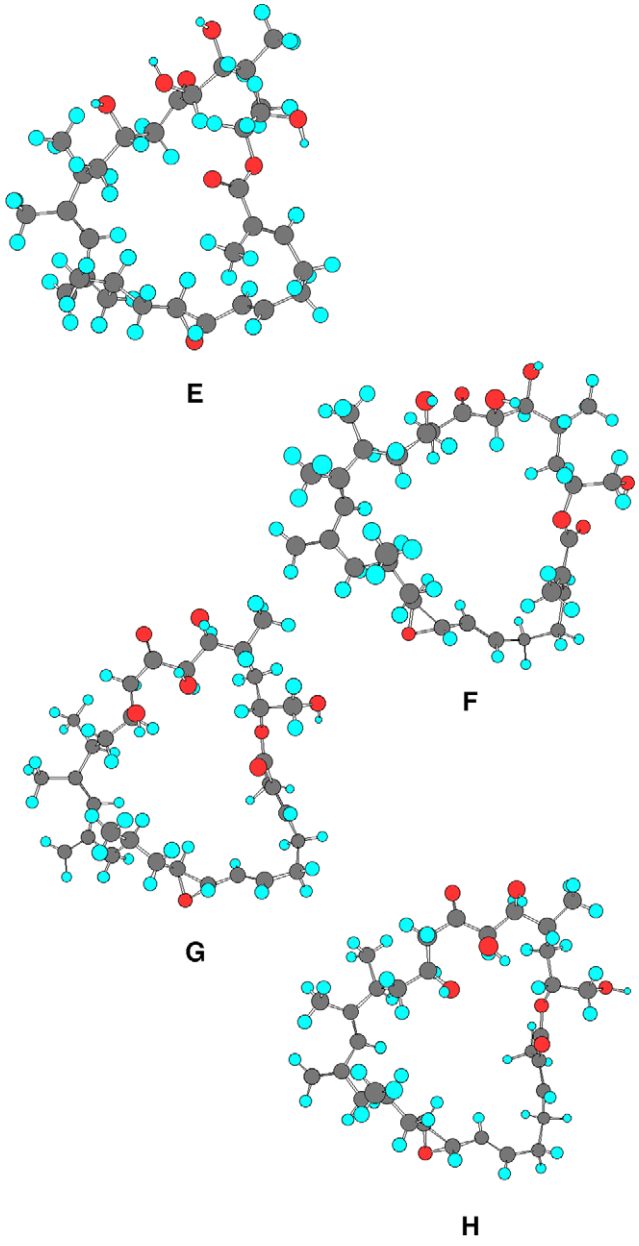


Figure 5. Low-energy conformations of the four confomational families **E**–**H** of amphidinolide H (**1**) calculated by distance geometry analysis based on ROESY data in DMSO-d_6.

(O7)H was suggested by their oxygen-oxygen distances [O4–O6: 2.62(3) Å, O5–O6: 3.32(2) Å, and O5–O7: 2.97(2) Å].

Three-dimensional conformations in $CDCl_3$ were suggested to be close to the X-ray structure of **1**, while the solution conformations in DMSO-d_6 were quite different from those in $CDCl_3$ and the X-ray conformation from the point of orientation of the epoxide oxygen atom (Fig. 7). In the conformations in DMSO-d_6, the epoxide oxygen atom was oriented outside of the macrocyclic ring. The conformational difference may be due to the polar, aprotic nature of DMSO. Hydrogen bonding in an intermolecular fashion may be responsible between the oxygen atom of sulfoxide and three hydroxyl groups in **1**.

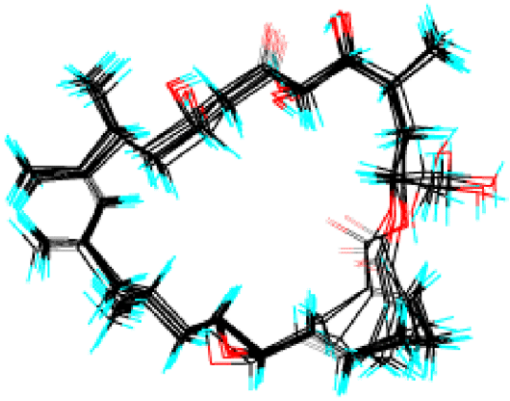


Figure 6. Overlay of the seven **E**-type conformations of amphidinolide H (**1**) calculated by distance geometry calculation in DMSO-d_6.

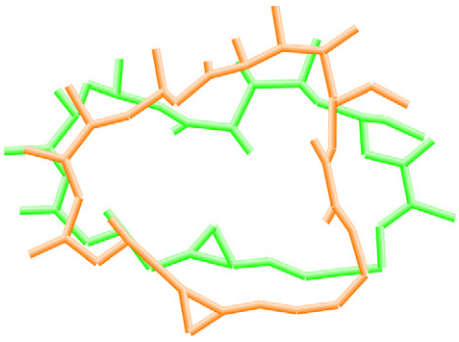
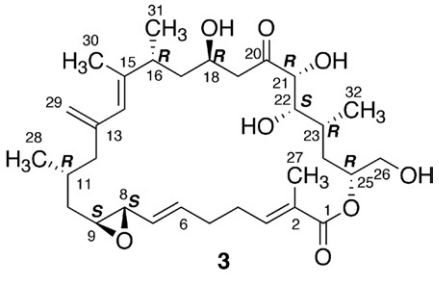


Figure 7. Overlay of the most stable conformation of amphidinolides H (**1**) in $CDCl_3$ (green) and DMSO-d_6 (orange).

In our previous studies for SAR of amphidinolide H (**1**), it was revealed that the presence of an allyl epoxide, an *S-cis*-diene moiety, and a ketone at C-20 was important for the cytotoxicity.[7] On the other hand, the 16,18-epimer of **1**, amphidinolide H2 (**3**), was 100 times less potent than that of **1**, although the most stable solution conformation of **3** in $CDCl_3$ was close to that of **1**



3

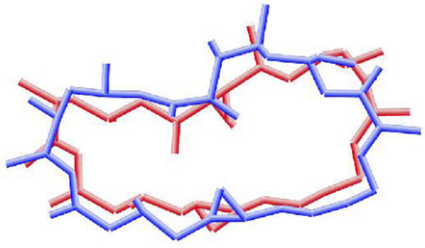


Figure 8. Overlay of the most stable conformation of amphidinolides H (**1**, blue) and H2 (**3**, red) in $CDCl_3$.

(Fig. 8). These suggest that the most stable conformation of **1** might not be responsible for potent cytotoxicity. Both the presence of some functional groups and relevant conformations of **1** may be important for the activity.

3. Experimental

3.1. NMR methods

NMR spectra were recorded on a 600 MHz spectrometer at 300 K using 1.0 mg sample of amphidinolide H (**1**) in 2.5 mm microcells for $CDCl_3$ and DMSO-d_6 (Shigemi Co., Ltd). The mixing time for NOESY spectra was set to 0.6 s. For ROESY spectra, all pulses were high-power output of the decoupler attenuated by 34 dB (the $\pi/2$ pulse duration was 100 μs, a 2500 Hz spin-lock field). The ROESY mixing was set to 0.25 s using spin-lock composed of two CW pulses (shifted in phase by positive and negative). NOESY and ROESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 64 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. Zero-filling was performed with squared cosine-bell windows in both the dimensions prior to 2 D Fourier transformation. The resulting data matrices for NOESY and ROESY were both 1K × 1K. The total measurement times for NOESY and ROESY were ca. 9 and 8 h, respectively. NOESY and ROESY spectra afforded useful 86 and 74 correlations, respectively.

3.2. Computational methods

Computer model and all calculations were carried out using the molecular-modeling software package SYBYL VER.6.5 (Tripos, Inc.) on a Silicon Graphics O2 computer. Distance geometry, simulated annealing, and molecular mechanics calculations were performed with Tripos 5.2 force field.[12] In the distance geometry calculation, interatomic distances were calculated from the integrated volumes of the 86 NOESY or 74 ROESY cross-peaks for amphidinolides H (**1**). The distance between H-24a and H-24b was set to 1.551 Å ± 20%. For each compound, 100 conformers were generated. In simulated annealing simulation, each conformation was equilibrated for 5000 fs in a thermal bath at 1000 K, and thereafter successively for 1450 fs, the temperature was deceased until a final structure of 300 K was reached, and each conformation was finally minimized.

Acknowledgments

This work was partly supported by a Grant-in-Aid from the Takeda Science Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References and notes

1. (a) Tsuda, M.; Izui, N.; Shimbo, K.; Sato, M.; Fukushi, E.; Kawabata, J.; Katsumata, K.; Horiguchi, T.; Kobayashi, J. *J. Org. Chem.* **2003**, *68*, 5339–5345, and reference cited therein; (b) Kobayashi, J.; Ishibashi, M. *Chem. Rev.* **1993**, *93*, 1753–1769; (c) Ishibashi, M.; Kobayashi, J. *Heterocycles* **1997**, *44*, 543–572.
2. Kobayashi, J.; Shigemori, H.; Ishibashi, M.; Yamasu, T.; Hirota, H.; Sasaki, T. *J. Org. Chem.* **1991**, *56*, 5221–5224.
3. Ishibashi, M.; Ohizumi, Y.; Hamashima, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1127–1129.
4. Bauer, I.; Maranda, L.; Shimizu, Y.; Peterson, R. W.; Cornell, L.; Steiner, J. R.; Clardy, J. *J. Am. Chem. Soc.* **1994**, *116*, 2657–2658.
5. Ishibashi, M.; Ishiyama, H.; Kobayashi, J. *Tetrahedron Lett.* **1994**, *35*, 8241–8242.
6. Kobayashi, J.; Shimbo, K.; Sato, M.; Shiro, M.; Tsuda, M. *Org. Lett.* **2000**, *2*, 2805–2807.
7. Kobayashi, J.; Shimbo, K.; Sato, M.; Tsuda, M. *J. Org. Chem.* **2002**, *67*, 6585–6592.
8. Tsuda, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1994**, *59*, 3734–3737.
9. Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *J. Nat. Prod.* **1989**, *52*, 1036–1041.
10. Usui, T.; Kazami, S.; Dohmae, N.; Mashimo, Y.; Kondo, H.; Tsuda, M.; Terasaki, G. A.; Ohashi, K.; Kobayashi, J.; Osada, H. *Chem. Biol.* **2004**, *11*, 1269–1277.
11. Matsunaga, K.; Nakatani, K.; Ishibashi, M.; Kobayashi, J.; Ohizumi, Y. *Biochim. Biophys. Acta* **1999**, *1427*, 24–32.
12. Clark, M.; Cramer, R. D., III; Van Opdenbosch, N. *J. Comput. Chem.* **1989**, *10*, 982–1012.